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Revealing Seasonal Dietary Niche Overlap Among Sympatric Large Carnivores using DNA Metabarcoding

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Abstract

Understanding how large carnivores partition dietary resources is essential for assessing intra-guild competition and informing conservation strategies. In this study, we used DNA metabarcoding of scats to quantify and compare the diets of sympatric African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) across wet and dry seasons in the Greater Etosha Landscape of Namibia. Across 98 scat samples (lion = 69; spotted hyena = 29), we identified 19 vertebrate prey species. Overall, large ungulates dominated both carnivores' diets. For lions, the most frequent prey items included gemsbok (*Oryx gazella*), common eland (*Taurotragus oryx*), plains zebra (*Equus quagga burchelli*), and blue wildebeest (*Connochaetus taurinus*). For spotted hyenas, the most frequent prey items were plains zebra, gemsbok, springbok (*Antidorcas marsupialis*), and black rhinoceros (*Diceros bicornis bicornis*). Dietary niche breadth was not significantly different between species, though lions exhibited the broadest across both seasons, while diet composition was similar between species and seasons. However, the smaller sample size for spotted hyenas may limit full characterization of their diet and influence measures of overlap and niche breadth. These results suggest a moderate diet overlap and limited resource partitioning both within and among these large carnivore species across seasons, likely facilitated by opportunistic scavenging and kleptoparasitism. Both species exhibited broader dietary niche breadths during the wet season, likely reflecting increased prey availability and dispersion. Ongoing monitoring of carnivore diets using molecular tools, which provides a more accurate and comprehensive identification of diet items than manual sorting, will be essential for detecting changes in resource use and interspecific interactions in response to shifting environmental conditions and anthropogenic pressures.

Keywords: African lions, diet composition, dietary niche breadth, interspecific competition, resource partitioning, spotted hyenas

Introduction

Large carnivores play critical roles in maintaining ecosystem health and diversity (del Rio et al., 2001). Yet, despite multifaceted conservation initiatives, large carnivore populations are declining globally due to anthropogenic pressures (Parmesan, 2006; Di Marco et al., 2014; Ripple et al., 2014) including human-mediated conflicts due to their large home range requirements and the expanding human footprint (Carbone and Gittleman, 2002). As population footprint expands, large carnivores are becoming increasingly restricted to protected areas (Mills and Harvey, 1998; Riggio et al., 2012), which can lead to higher levels of interspecific interactions and intra-guild competition. Thus, monitoring large carnivore populations, especially where intra-guild competition is likely to be high, is imperative for conservation efforts (Linnell and Strand, 2000; Burgar et al., 2019).

In regions with well-preserved predator guilds, large carnivore species often occupy overlapping geographic ranges and compete for prey resources. Interspecific competition can drive community structure and function, and affect the distribution, demographic patterns, and behavioral strategies of co-occurring species (Chase et al. 2002, Chesson and Kuang 2008, Vanak et al., 2013). Because large carnivore populations are primarily shaped by the abundance and availability of their main food items (Karanth et al., 2004; Hayward & Kerley, 2008), prey represents a critical resource that coexisting carnivores can partition to reduce competition (Schoener, 1983; Hayward &

Kerley, 2008). Intra-guild competition intensity can vary spatio-temporally due to changes in prey availability, which can fluctuate due to a multitude of biotic and abiotic factors such as seasonality, droughts, climate change, or disease outbreaks (Walther et al., 2002; Duncan et al., 2012; Zidon et al., 2017). While numerous studies have investigated carnivore competition and resource partitioning, data is lacking in certain areas of sub-Saharan Africa, where largely intact carnivore guilds exist. Understanding how sympatric carnivores partition dietary resources in these systems is thus essential to understand carnivore ecology (Radloff and du Toit, 2004) and inform conservation practices through vegetation and prey species management.

Some of the most diverse and multifaceted terrestrial assemblages of large carnivores occur in sub-Saharan Africa, with several species of large carnivores occurring sympatrically including African lions (*Panthera leo*) (hereafter lions), spotted hyenas (*Crocuta Crocuta*), cheetahs (*Acinonyx jubatus*), leopards (*Panthera pardus*), and brown hyenas (*Parahyaena brunnea*). Lions and spotted hyenas are two of the most numerous large carnivores in this region where they overlap extensively in distribution (Hatton et al., 2015) and diel activity (Mills & Biggs, 1993; Hayward & Hayward, 2007; Patterson et al., 2024). To facilitate coexistence, lions and spotted hyenas have evolved disparate behavioral, social, and foraging strategies (Périquet et al., 2015; Swanson et al., 2016). Lions are ambush predators, who hide under vegetation and pursue medium-large ungulate prey (Hayward and Kerley, 2005; Périquet et al., 2015), yet also hunt in open areas (Stander, 1992) and scavenge opportunistically (Moleón et al., 2015; Amorós et al., 2020). Conversely, spotted hyenas are cursorial predators, typically chasing prey and using endurance for hunting success (Kruuk, 1972; Mills, 1990) but also readily scavenge carcasses when available (Watts & Holekamp, 2009; Moleón et al., 2015). Additionally, spotted hyenas do successfully hunt calves or juvenile large ungulate species (Kruuk, 1972; Holekamp et al., 1997; Salnicki et al., 2001).

Lions and spotted hyenas engage in kleptoparasitism; spotted hyenas lose more of their kills to lions than lions lose to spotted hyenas (Périquet et al., 2015), though this kind of intra-guild competition is highly variable based on pride and clan size and structure (Trinkel and Kastberger, 2005). With overlapping diel activity patterns, kleptoparasitism, and opportunistic scavenging, dietary overlap is expected to be high between lions and spotted hyenas with substantial variation across their shared geographic range. Previous studies report broad dietary niches for spotted hyenas (Vissia et al., 2023), with similar (Hayward, 2006) or narrower (Hayward & Kerley, 2008) niche breadths compared to lions. However, spotted hyenas may reduce their niche overlap by preying on different prey size via different sex or age classes (Périquet et al., 2015). Additionally, top prey items vary across studies for both species (e.g., Hayward, 2006), and the role of seasonality in shaping dietary niche breadth and overlap remains poorly understood (Vissia et al., 2023).

Few studies have employed high-throughput sequencing methods like DNA metabarcoding to address these questions. DNA metabarcoding has been shown to have higher sensitivity and greater taxonomic resolution relative to conventional dietary studies that rely on morphological identification of undigested remains in scats (Stein et al., 2014; Galan et al., 2017). Morphological identification can introduce additional biases into dietary results through misidentification of closely related prey items, lack of detection for prey items with highly digestible tissues, and underestimating prey items (Monterosso et al., 2019). The conflicting findings in previous dietary studies, along with the limited application of molecular techniques, underscore the importance of assessing carnivore diet partitioning, especially between lions and spotted hyenas, to quantify dietary overlap in a rich carnivore community. Moreover, while little is known about the effects of seasonality on dietary niche overlap among large carnivores, understanding these dynamics is critical for advancing our knowledge of intra-guild resource partitioning and coexistence in shared landscapes.

Our goal in this study was to use DNA metabarcoding to quantify dietary patterns of sympatric lions and spotted hyenas in a dryland ecosystem in southern Africa. Specifically, our objectives were to 1) quantify the vertebrate diet composition of sympatric lions and spotted hyenas; 2) quantify seasonal variation in diet composition within and between each species; 3) assess dietary niche breadth for each species across seasons; and 4) evaluate interspecific dietary overlap to determine if there is evidence of niche partitioning and competition. We also formulated a series of predictions. First, we predicted that both species would have higher diversity in their diet during the wet season due to increased availability of young herbivores during the birthing season for many ungulate species, and animals dispersing across the landscape and away from predictable waterhole locations, forcing carnivores to be less selective for prey species. Second, based on scavenging activity and previous studies (Hayward, 2006; Vissia et al., 2023), we predicted that spotted hyenas would have higher dietary niche breadths than lions during both seasons. Lastly, we predicted that both species would have high dietary overlap during both seasons because of kleptoparasitism and opportunistic scavenging.

Methods

Study Area

This study was conducted in the Greater Etosha Landscape (GEL) encompassing Etosha National Park (“Etosha”), the Ongava Game Reserve (“Ongava”), and King Nehale communal conservancy (“King Nehale”) in north-central Namibia (Fig. 1) (Turner et al., 2022). Etosha is a 22,900 km^2 fenced protected area, although the fence is permeable to wildlife (Naha et al., 2023; Melton, 2024). Ongava is a fenced 300 km^2 private game reserve on the southern border of Etosha. King Nehale is a 508 km^2 communal conservancy located in the Oshikoto region, bordering the northeast corner of Etosha. One main source of income for people living in the conservancy is keeping livestock, including cattle, goats, and sheep (Nakanyala et al., 2022). The GEL is located within the semi-arid weather zone with an average annual rainfall of 380 mm (De Beer et al., 2006; Atlas of Namibia, 2022). Despite fencing around Etosha, the prey and predator guild composition in all these areas is very similar and together they form part of the same larger scale ecosystem, including movement of carnivores between Ongava and Etosha, and more so between King Nehale and Etosha (Melton, 2024). The main vegetation types in Etosha are grasslands, steppe, shrubland, Mopane (*Colophospermum mopane*) tree savanna, and mixed tree savanna (Le Roux et al., 1988). The landcover on Ongava is classified as Karstveld, with Mopane shrubland and woodland being the dominant vegetation type (~70%) and other areas with savannah-like vegetation (~30%, Berry and Loutit, 2002). During the wet season (November-April), rainfall provides additional temporal water sources, aside from the permanent artificial water sources across the landscape (Engert, 1997; Berezin et al., 2023).

Both Etosha and Ongava are tourist destinations for wildlife-based viewing and support a broad diversity of carnivore and ungulate species [e.g., black-faced impala (*Aepyceros melampus petersi*), springbok (*Antidorcas marsupialis*), blue wildebeest (*Connochaetus taurinus*), plains zebra (*Equus quagga burchelli*), gemsbok (*Oryx gazella*)], including megaherbivores [e.g, African elephant (*Loxodonta africana*), southern Angolan giraffe (*Giraffa giraffa angolensis*), southern black rhinoceros (*Diceros bicornis bicornis*)]. However, prey species' composition and abundance differ between Ongava and Etosha, and across the different regions of Etosha (west, central, and east) (Trinkel, 2010; Kilian and Kolberg, 2015). Across both sites, potential prey species richness for mammals >5kg is approximately 21 wild species and three domestic livestock species. In 2008, the estimated spotted hyena population in Etosha was 339 ± 176 (Trinkel, 2009) and in 2022, the estimated lion population in Etosha was 350 (MEFT, 2022). Data are unavailable for population sizes in Ongava.

Sample Collection

We collected 164 dried or fresh fecal samples [spotted hyena (n=33) and lion (n=132)] from October 2021 through March 2024 around Etosha, Ongava, and King Nehale. When divided by location, we collected 74 samples in Etosha [spotted hyena (n=28; dry season = 20, wet season = 8) and lion (n=46; dry season = 40, wet season = 6)], three samples in King Nehale [spotted hyena (n=2 in the wet season) and lion (n=1 in the dry season)], and 88 samples in Ongava [spotted hyena (n=3 in the dry season) and lion (n=85; dry season = 77, wet season = 8)]. Most samples (n = 140) were collected opportunistically from the ground while driving along roads, around waterholes, and near carnivore kill sites within our study area (approved by Namibian Ministry of Environment, Forestry, and Tourism under permit #AN202101004). For these samples, whole fecal samples were collected into a paper bag and dried naturally before being subsampled for DNA extraction, which included both the external surface and internal matrix to capture both consumer and prey DNA. Additionally, 24 samples [lions (n=19), spotted hyenas (n=5)] were collected during carnivore immobilizations as part of ongoing research (Appendix S1; Text S1). These samples were manually retrieved from the immobilized animal, placed into an open plastic vial and stored in a paper bag for natural drying. Due to the small amount collected directly from immobilized animals, these samples were not subsampled. All immobilizations were performed by veterinarians registered with the Namibian Veterinary Council and the Ministry of Environment, Forestry, and Tourism and were approved by the Namibian Ministry of Environment, Forestry, and Tourism (permit #AN202101004) as well as the University of Georgia Institutional Animal Care And Use Committee under protocols A2024 05-009-06 and A2021 04-013-Y3-A11.

Laboratory Analyses and Data Curation

All samples were shipped from Namibia to the United States in July 2024 and DNA was extracted from feces using DNeasy PowerSoil Pro DNA Kits (QIAGEN) in August 2025. Jonah Ventures, LLC conducted DNA amplification, sequencing, and bioinformatics for DNA metabarcoding (Appendix 1; Text S2). This technique analyzes DNA within scats using high-throughput sequencing using small, highly variable universal primers to identify prey species and confirm predator species (Pompanon et al., 2012; Xiong et al., 2017). To characterize prey DNA from carnivore fecal samples, we amplified a portion of the 12S rRNA gene using the primer pair 12SVertF (forward 5′- ACTGGGATTAGATACCCYACTATG -3′) and 12SVertR (reverse 5′- GAGRRTGACGGGCGGTDT -3′) (Evans et al., 2016). Both forward and reverse primers contained a 5’ adaptor sequence to allow for subsequent indexing and Illumina sequencing. Exact sequence variants (ESVs) were identified using the UNOISE3 algorithm (Edgar, 2016a) implemented in VSEARCH (Rogens et al., 2016), retaining only those with ≥ 4 read counts and ≤ 1 expected error. We assigned taxa of ESVs using a custom reference database consisting of publicly available sequences on NCBI GenBank (Benson et al., 2005) and Jonah Ventures voucher sequences records, accepting the reference taxonomy for any taxonomic level with > 90% agreement within 1% of the top hits. We discarded any reads of taxa not occurring in the study region, and to limit the effects of contamination, only prey species that consisted of more than 1% of total prey sequences were retained (Shively et al., 2025). Because host DNA typically dominates the sequencing dataset (Forin-Wiart et al., 2018), we were able to confirm predator species for each sample.

Data Analyses

We conducted all analyses and produced figures using R version 4.1.1 (R Core Team, 2024). We summarized the data with both frequency of occurrence (FOO) and relative read abundance (RRA) at the species level, and calculated prey species richness for each carnivore species by season (wet and dry). To calculate FOO, we divided the number of occurrences of a prey species by the total number of carnivore scat samples and expressed FOO as a percentage. We calculated RRA for each prey species as the read count (the number of DNA sequence reads) for that the given prey species divided by the total number of reads in the carnivore scat sample, also expressed as a percentage.

To evaluate differences in vertebrate prey composition (beta diversity) between carnivore species and seasons, we performed non-metric multidimensional scaling (NMDS). Analyses were conducted separately for wet and dry seasons using Bray–Curtis dissimilarities derived from binary presence–absence matrices, where each sample with identified prey was represented by a vector of prey species detections. NMDS ordinations were computed in three dimensions (k = 3) with 1000 permutations (trymax = 1000) to improve solution stability, and we used stress values assess fit. We generated plots where arrows represent prey species most associated with carnivore-specific patterns in prey use and samples cluster apart in ordination space if carnivore diets are compositionally distinct.

We used two complementary approaches to evaluate dietary similarity between and within carnivore species. First, we applied a non-parametric Analysis of Similarities (ANOSIM) with binary Jaccard dissimilarities to test whether prey composition within carnivore scats differed between lions and hyenas within each season. Because ANOSIM evaluates overall compositional differences but does not quantify the degree of overlap, we also calculated

Pianka's index of niche overlap (Pianka, 1973) using FOO, with values ranging from 0 (no overlap) to 1 (complete overlap) based on the proportional use of each prey species. To account for unequal sample sizes, we generated 95% bootstrap confidence intervals by stratified resampling of carnivore scats for both species (2,000 iterations). Together, these analyses allowed us to assess both whether diets differed significantly between carnivore species and the extent of dietary niche overlap, providing a more complete picture of dietary similarity across seasons. We used the vegan package in R (Oksanen et al., 2025) to conduct the NMDS and ANOSIM and spaa package in R (Zhang, 2016) to determine Pianka's index.

We calculated dietary niche breadth (*Ba*) for each carnivore species in both seasons by using Hurlbert's standardized version of Levin's measure (Hurlbert, 1978; Smith, 1982) for FOO proportions of prey. To account for differences in sample size across carnivore species and seasons, we applied a bootstrap approach with 2,000 iterations. For each iteration, all samples were resampled with replacement within each carnivore species–season group, and standardized niche breadth was recalculated. Broader dietary niche breadth is indicated by a higher *Ba* while a narrow dietary niche breadth is indicated by a low *Ba* (ranging from 0-1). We did this overall with all data pooled, and separately for each study site [Etosha (including King Nehale) versus Ongava].

To evaluate completeness of our sampling efforts and prey richness, we generated sample-based rarefaction and extrapolation curves for each carnivore species and season using the specaccum function in the vegan package (Oksanen et al., 2022). Scat samples were converted to presence–absence matrices of prey taxa, and 1,000 random permutations were used to estimate mean prey richness and associated 95% confidence intervals. We further estimated asymptotic prey species richness with the Chao1 estimator (Chao, 1984; specpool function), a non-parametric approach that accounts for incomplete sampling by inferring the total number of species based on the frequency of rare species observed only once or twice. We then plotted these estimates (± standard error) alongside rarefaction curves to assess whether observed prey detections approached estimated richness. For all analyses we pooled data from all study sites to generate broad inferences about carnivore diet, and we also visually examined diet composition between Etosha and King Nehale versus Ongava.

Results

Diet overview

From the 164 samples that were sequenced, 98 had detectable vertebrate prey items and were included in analysis [spotted hyena (n=29; dry season=17, wet season=12) and lion (n=69; dry season=54, wet season=15)]. Overall, we detected 9 families and 17 genera encompassing 19 vertebrate prey species (Table 1). For spotted hyenas, the total read count was 127640 with a mean read count of 3868 per sample (± 4461 SD). For lions, the total read count was 107965 with a mean read count of 1367 per sample (± 2733 SD).

Spotted hyena diet

From 29 sequenced spotted hyena samples, plains zebra was the most frequently consumed prey species in the dry season (FOO = 29.4%) followed by gemsbok (*Oryx gazella*) and springbok (FOO = 17.7% each). In the wet season, spotted hyenas equally consumed plains zebras and gemsbok (FOO = 18.8% each, Fig. 2). Overall, prey species richness was 10 and included two domestic livestock species, cow (*Bos taurus*; n=2) and goat (*Capra hircus*; n=2). Both domestic prey species were found in samples near or outside of northeast Etosha (Fig. 1). ANOSIM revealed no seasonal differences in diet composition for spotted hyenas (Table 2).

When we separated out by study site [Etosha and King Nehale (n=30; dry season=20, wet season=10) versus Ongava (n=3 in the dry season)], during the dry season, plains zebra (FOO = 33%) and springbok (FOO = 20%) were the most consumed prey items in Etosha (Fig. S1) while gemsbok and kudu (*Tragelaphus strepsiceros*) were the only consumed prey items in Ongava (FOO = 50% each) (Fig. S2). During the wet season, spotted hyenas primarily consumed plains zebra and gemsbok in Etosha (FOO = 25% each) (Fig. S1).

Lion diet

From 69 sequenced samples, lions consumed a wide variety of species but primarily large ungulates such as gemsbok (FOO = 25.4%), followed by blue wildebeest and eland (*Taurotragus oryx*) (FOO = 13.6% each), then plains zebra (FOO = 10.2%) during the dry season. In the wet season lions consumed eland, gemsbok, and plains zebra equally (FOO = 15% each), followed by black-faced impala, blue wildebeest, giraffe, and hare species (FOO = 10% each, Fig. 2). Overall, prey richness was 16 and ANOSIM revealed no significant seasonal differences in diet composition for lions (Table 2).

Separated out by study site [Etosha and King Nehale (n=47; dry season=41, wet season=6) versus Ongava (n=85; dry season=77, wet season=8)], during the dry season, lions consumed springbok (FOO = 21%) most frequently in Etosha, followed by giraffe, plains zebra, eland, red hartebeest, and black rhinoceros equally (FOO = 12%) (Fig. S1). During the dry season on Ongava, lions consumed gemsbok most frequently (FOO = 35%), followed by blue wildebeest (FOO = 20%), eland (FOO = 15%), and plains zebra (FOO = 10%) (Fig. S2). During the wet season in Etosha, lions primarily consumed giraffe and plains zebra equally (FOO = 28%) followed by eland, red hartebeest, blue wildebeest, kudu, and python species equally (FOO = 14%) (Fig. S1). During the wet season on Ongava, lions consumed gemsbok, eland, black-faced impala, and hare spp. equally (FOO = 25%), followed by blue wildebeest and plains zebra (FOO = 12.5%) (Fig. S2).

Dietary niche breadth

Overall, during the wet and dry seasons, lions exhibited broader dietary niche breadth [*Ba*: wet = 0.45 (0.27-0.54); dry = 0.4 (0.32-0.56)] than spotted hyenas [*Ba:* wet = 0.38 (0.21-0.48)]; dry = 0.28 (0.15-0.39)] though there were no significant differences between species. Both species had broader dietary niche breadths during the wet season compared to the dry season (Fig. 3), with no significant differences between seasons for either species. In Etosha, lions exhibited broader dietary niche breadth than spotted hyenas in the dry season (*Ba:* lions = 0.5, hyenas = 0.29), but spotted hyenas had slightly broader dietary niche breadths in the wet season (*Ba:* lions = 0.44, hyenas = 0.47). Lions had broader dietary niche breadths in the dry season, while spotted hyenas were broader in the wet season (Fig. S3). In Ongava, lions had similar dietary niche breadths across seasons (*Ba:* dry = 0.417, wet = 0.414), and broader dietary niche breadth compared to spotted hyenas in the dry season (*Ba* = 0.09) (Fig. S4).

Interspecific dietary variation

We found no differences in dietary composition for either carnivore species overall (all data pooled) or across seasons. The NMDS ordination for dietary overlap (Fig. 4) had a stress value of 0 in the wet season and <0.001

in the dry season. While a stress value of or near 0 is uncommon, it can occur in small or low-dimensional datasets but is under the threshold value (stress < 0.2) as suggested by Clarke (1993) for an interpretable ordination. Pairwise ANOSIM comparisons revealed no significant differences ($p > 0.05$) in diet composition between lions and spotted hyenas within either season or overall. R statistics were close to zero or negative, indicating strong dietary overlap among these carnivores across seasons and overall (Table 2). Pianka's index revealed moderate dietary overlap between lions and spotted hyenas overall ($O = 0.75$). Seasonal analyses showed that overlap between species was similar between the dry season ($O = 0.68$) and the wet season ($O = 0.66$). Within species, lions exhibited greater dietary similarity across wet and dry seasons ($O = 0.84$) compared to spotted hyenas ($O = 0.77$) (Table 3).

Rarefaction curves

Rarefaction curves revealed that prey species richness in lion scats approached asymptotic levels in the dry season, where observed richness (15 taxa) closely matched Chao1 estimates (17.0 ± 2.6). In the wet season, lion richness was lower (10 taxa, 11.0 ± 1.7), suggesting some rare prey were likely not captured. Spotted hyena curves indicated lower observed richness overall (dry = 8, wet = 9 taxa), with estimates suggesting additional undetected prey species, particularly in the dry season (Chao1 = 15.5 ± 11.0), though uncertainty was high due to small sample size (Fig. 5).

Discussion

Determining how large carnivores partition dietary resources is critical for understanding intra-guild interactions and coexistence. Using DNA metabarcoding, we characterized the diets of lions and spotted hyenas in the GEL and found substantial dietary similarity between both species, regardless of the season. Despite differences in prey species richness and niche breadth between carnivore diets, there were no significant seasonal or interspecific differences in prey species composition. These findings suggest that in this landscape, diet composition alone may not strongly differentiate resource use between lions and spotted hyenas, and that any niche partitioning may occur through prey age or sex selection, spatial segregation, or other behavioral mechanisms not captured by prey identity alone. Collectively, these data contribute to our overall understanding of dietary patterns of sympatric lions and spotted hyenas in a semi-arid ecosystem.

Species with broad dietary niche breadths are typically considered dietary generalists while those with narrow dietary niche breadths are considered specialists (Carvalho & Cardoso, 2020). In our study, lions had a wider dietary niche breadth during both seasons compared to spotted hyenas; however, our findings demonstrate that lions and spotted hyenas exhibit broadly similar diets with consistently high overlap, suggesting that these two apex predators rely heavily on a shared prey base. When comparing dietary niche breadth between species, lions and spotted hyenas are reported to have similar dietary niche breadths, which aligns with our results (Hayward, 2006; Hayward and Kerley, 2008, reviewed in Périquet et al., 2015). The comparison between seasons supported our prediction that both lions and spotted hyenas had broader dietary niche breadths in the wet season compared to the dry. This difference in seasonality has been previously documented in African mammal diets (Vissia et al., 2023) perhaps because some prey species are more abundant and more easily accessible (Trinkel, 2013; Pereira et al., 2014) during the birthing season or because rainfall leads to prey dispersing across the landscape. As a result, predators can no longer rely on high prey densities near predictable waterhole locations and may be less selective, consuming whatever prey they encounter. However, because spotted hyena sample sizes were lower than those for lions, estimates of hyena dietary niche breadth should be interpreted with caution.

The results are consistent with moderate dietary overlap between both carnivore species, as indicated by Pianka's index (O = 0.75). This pattern was generally similar across seasons, with only minor fluctuations between wet and dry periods. Apart from domestic livestock species, all prey species detected in the spotted hyena diet were also found in the lion diet. Extensive dietary overlap may indicate occurrences of kleptoparasitism by lions and spotted hyenas, which previously have been reported (Trinkel and Kastberger, 2005). Additionally, lions and spotted hyenas are documented competitors that consume similar prey items (Hayward, 2006; Hayward and Kerley, 2008) and exhibit comparable temporal activity patterns (Mills & Biggs, 1993; Hayward & Hayward, 2007; Patterson et al., 2024). Lions and spotted hyenas have been documented partitioning spatially (Evers et al., 2022; Patterson et al., 2025), though some studies have found a high degree of spatial overlap (Périquet et al., 2015; Swanson et al., 2016). Further, hunting styles can differ between the two predators; lions conceal behind vegetation to pursue prey (Périquet et al., 2015), yet also hunt in open areas (Stander, 1992), while spotted hyenas typically chase prey in more open areas (Kruuk, 1972; Mills, 1990) but also readily scavenge carcasses when available (Watts & Holekamp, 2009; Moleón et al., 2015). Thus, our findings indicate that lions and spotted hyenas in Etosha may partition their prey resources by age or sex class, or spatially to co-exist and we recommend further studies to quantify prey partitioning across demographic classes and spatial scales to better understand mechanisms of niche differentiation and coexistence among large carnivores.

In a review of spotted hyena diet across several countries, Hayward (2006) determined that gemsbok was the most frequently consumed prey species where it occurred, and plains zebra and springbok were typically consumed infrequently. Conversely, other studies (Gasaway et al., 1991; Trinkel, 2010) found plains zebra and springbok were the main prey species for spotted hyenas in some areas of Etosha, while Berry (1981) determined wildebeest and plains zebra were the main prey species in Etosha. Fester et al. (2021) found gemsbok and springbok were the main prey items in the Namib desert of southwest Namibia. In our study, spotted hyenas most frequently consumed plains zebras, gemsbok, and springbok, respectively. As such, spotted hyenas may target gemsbok but also consume plains zebras and springbok in the GEL due to their high abundance and availability compared to other ungulates. For example, in 2015, the Etosha density of gemsbok was estimated at 0.22 individuals/km^2 while plains zebra was 0.77 individuals/km^2 and springbok were 0.56 individuals/km^2 (Kilian and Kolberg, 2015). As plains zebra and springbok are grazers (Kingdon, 2015), and gemsbok are mixed feeders that primarily graze (Sponheimer et al., 2003; Lehmann et al., 2020), these results also align with Patterson et al. (2025) who found that spotted hyenas in Etosha select for areas of high grass cover during both seasons. Collectively, these studies indicate that in the GEL, spotted hyenas are likely hunting in open, grassy areas and primarily consuming grazing prey species.

The range of prey items for lions showed no strong selection for either browsers or grazers, as both (browsers: giraffe; mixed: eland, gemsbok; grazers: plains zebra, blue wildebeest, Kingdon, 2015) were found as lion top prey species. Our results align with findings from Hayward and Kerley (2005) who found lions' primary prey species were gemsbok, blue wildebeest, giraffe, eland, and zebra across several countries and Berry (1981) who found lions' primary prey items in Etosha were gemsbok, wildebeest, and plains zebra. Consistent with other findings, our study shows that lions likely consume small prey opportunistically (Hayward and Kerley, 2005; Davidson et al., 2013; Barnardo et al., 2020). This supports the optimal foraging theory, which suggests that a predator can distinguish among prey of differing profitability and typically select the most profitable types (Krebs, 1978). Large-bodied grazers were consumed more during the dry season in our study, which may indicate that lions were opting for higher caloric returns from large prey species to sustain themselves during higher periods of stress (Hayward and Kerley, 2005). Additionally, in the dry season herbivores congregate at fixed waterhole locations in the GEL, increasing predator access to a higher density of potential prey. Conversely, in the wet season lions appear to consume fewer species, with several at higher frequencies relative to the dry season (e.g., hare spp., black-faced impala, giraffe), potentially reflecting opportunistic foraging or scavenging due to greater prey dispersion.

We found three spotted hyena samples with domestic livestock species (cow and goat). All samples were collected near or outside of Etosha in the northeast area (King Nehale communal conservancy) where livestock are abundant and spotted hyenas often leave the park and enter onto communal land (Naha et al., 2023; Melton, 2024; Patterson et al., 2025), sometimes resulting in retaliatory killings of carnivores after livestock depredation events (Stander, 2004; Goelst, 2018, Naha et al. 2025). Unfortunately, there is little information on the extent and demography of spotted hyenas killed in anthropogenic areas surrounding Etosha, but local farmers report frequent sightings of spotted hyenas on their farms (Lendelvo et al., 2019). No lion samples contained livestock species; while livestock depredation by lions has been reported around Etosha (Goelst, 2018), they are less likely to leave the park and cross the fence relative to spotted hyenas (Naha et al., 2023; Melton, 2024) into King Nehale. Instead, the southern periphery of Etosha is a more common area for human-lion conflicts and livestock depredation events by lions (Trinkel et al., 2016; Goelst, 2018) where no samples were collected for this study. These findings contribute to our overall understanding of livestock depredation in a high human-carnivore conflict area.

The rarefaction analyses highlight important differences in sampling completeness between lions and spotted hyenas, which help contextualize niche overlap between lions and spotted hyenas, as well as niche breadth estimates. We could not detect prey items in 66 samples due to predator DNA swamping, which occurs when host DNA is present in much higher concentrations than prey DNA (Shi et al., 2021). Host DNA swamping was especially prevalent in lion samples. While degradation of scat samples before collection can increase host DNA dominance (Oehm et al., 2011; Thuo et al., 2019), both lion and spotted hyena samples were primarily collected opportunistically, and exact scat age was unknown. Thus, differences in digestive physiology and feeding behavior may influence host DNA amplification bias and contribute to interspecific variation in prey detection rates (Cuff et al., 2023). Further research is needed to assess how such physiological and behavioral traits affect host DNA swamping and prey DNA degradation in dietary metabarcoding studies, particularly among apex carnivores like lions and spotted hyenas. Additionally, future metabarcoding studies may improve prey detection by explicitly optimizing sequencing depth, particularly for rare or highly degraded prey DNA. For lions, dry-season rarefaction curves approached asymptotic levels, and Chao1 estimates suggested that only a small number of prey species were likely undetected, indicating that our dietary characterization for lions is robust and provides a reliable picture of dry season prey use. In contrast, the rarefaction curve for spotted hyenas showed lower observed richness and substantially higher Chao1 estimates, particularly in the dry season where uncertainty in prey species detection was large, reflecting potential under-sampling. This limitation suggests that while lions and spotted hyenas appear to have high dietary overlap, the true extent of spotted hyena prey use may be broader than captured in this study. Of the 24 potential mammalian prey species in the system >5kg, lions consumed 13 and hyenas consumed 10, indicating that both predators utilized only a subset of the available prey community. Consequently, the high dietary overlap we observed may partially reflect more complete sampling of lion diets compared to spotted hyenas. Nonetheless, the combination of dietary niche overlap and breadth results indicates that both species rely heavily on shared prey resources, with lions exhibiting more stable and well-characterized prey use across seasons. Future work that includes expanded spotted hyena sampling could help clarify whether spotted hyena dietary breadth converges more strongly with or diverges from that of lions under different seasonal conditions.

We found notable differences in frequently consumed prey species and dietary niche breadth for both lions and spotted hyenas between study sites, likely reflecting variation in prey abundance and community composition within the sampled areas. Although both Etosha and Ongava are protected areas, they are managed by different entities and are different sizes, which may influence wildlife distributions and prey availability. However, the small number of spotted hyena samples from Ongava (n=2) limited our ability to accurately characterize their dietary niche breadth at that site. Additionally, because sampling did not encompass the entire extent of Etosha, we were unable to provide a comprehensive synthesis of lion and spotted hyena diets across the entire GEL. Despite these limitations, this study provides valuable insights into the diets of lions and spotted hyenas where dietary data, particularly derived from high-resolution DNA metabarcoding, are lacking.

Our study contributes to the area of emerging research using DNA metabarcoding analysis to compare diets of large, sympatric carnivores. The results indicate considerable dietary similarity between lions and spotted hyenas across both wet and dry seasons, though limitations in sample size, particularly for spotted hyenas, preclude strong conclusions about the extent of dietary overlap or niche partitioning. Our findings suggest that prey availability and opportunistic feeding behavior may be the primary drivers of diet composition in this well-preserved carnivore guild. Despite differences in dietary niche breadth, both species showed high overlap in key prey taxa, perhaps due to opportunistic scavenging and kleptoparasitism. These findings emphasize the importance of maintaining abundant ungulate populations to support diverse predator communities. Importantly, the detection of domestic livestock in spotted hyena diets highlights the potential for human–wildlife conflict, emphasizing the need for continued management interventions around Etosha. Further diet monitoring is essential for detecting shifts in predator–prey dynamics in response to environmental change, prey population fluctuations, or increased anthropogenic pressures. Future research should also explore the spatial and temporal aspects of scavenging and interspecific interactions to better inform adaptive management and conservation strategies in protected areas and surrounding multi-use landscapes.

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Statements and Declarations

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Data availability: The datasets generated and analysed during the current study are available in the NCBI Sequence Read Archive repository at https://www.ncbi.nlm.nih.gov/sra/PRJNA1357869.

Fig. 1 Location of collected fecal samples from African lions (*Panthera leo*, red dots) and spotted hyenas (*Crocuta Crocuta*, black dots) in the Greater Etosha Landscape, Namibia. Collection occurred between October 2021 and March 2024

Fig. 2 Seasonal composition of vertebrate prey species detected in the diets of African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) in the Greater Etosha Landscape based on DNA metabarcoding. Bars represent the proportion of fecal samples in which each prey species was detected, calculated as the number of unique samples per species and season divided by the total number of samples for that carnivore and season. Results are shown separately for the dry season (green) and wet season (grey), and prey species are ordered by overall frequency of occurrence from left to right. Only prey species with ≥1% relative read abundance were included

Fig. 3 Seasonal variation in standardized niche breadth (Hurlbert’s B′) for African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) in the Greater Etosha Landscape, in both the dry (May-October) and wet (November-April) seasons. Bars represent mean dietary breadth based on frequency of occurrence (FOO) of prey species in fecal samples, with error bars showing 95% confidence intervals from 2,000 bootstrap iterations

Fig. 4 Non-metric multidimensional scaling plot showing beta diversity in vertebrate prey species (12S rRNA) detected in the diet of African lions (*Panthera leo*), and spotted hyenas (*Crocuta crocuta*) in the Greater Etosha Landscape, by season (dry = May-October and wet = November-April). Points represent individual predator scat samples. The position of each point in ordination space reflects the multivariate composition of prey in that sample, with closer points indicating more similar prey profiles. Black arrows represent the direction and strength of correlation between specific prey species and the NMDS axes. Species labels at the arrow tips indicate which prey taxa most strongly influence variation in carnivore diet composition along NMDS axes

Fig. 5 Sample-based rarefaction and extrapolation curves of prey species richness detected in lion (*Panthera leo*) and spotted hyena (*Crocuta crocuta*) scat samples during dry (green) and wet (black) seasons. Solid lines represent mean richness estimates with shaded areas indicating 95% confidence intervals based on 1,000 random permutations. Triangles show observed richness, and vertical error bars represent Chao1 asymptotic richness estimates (± SE)

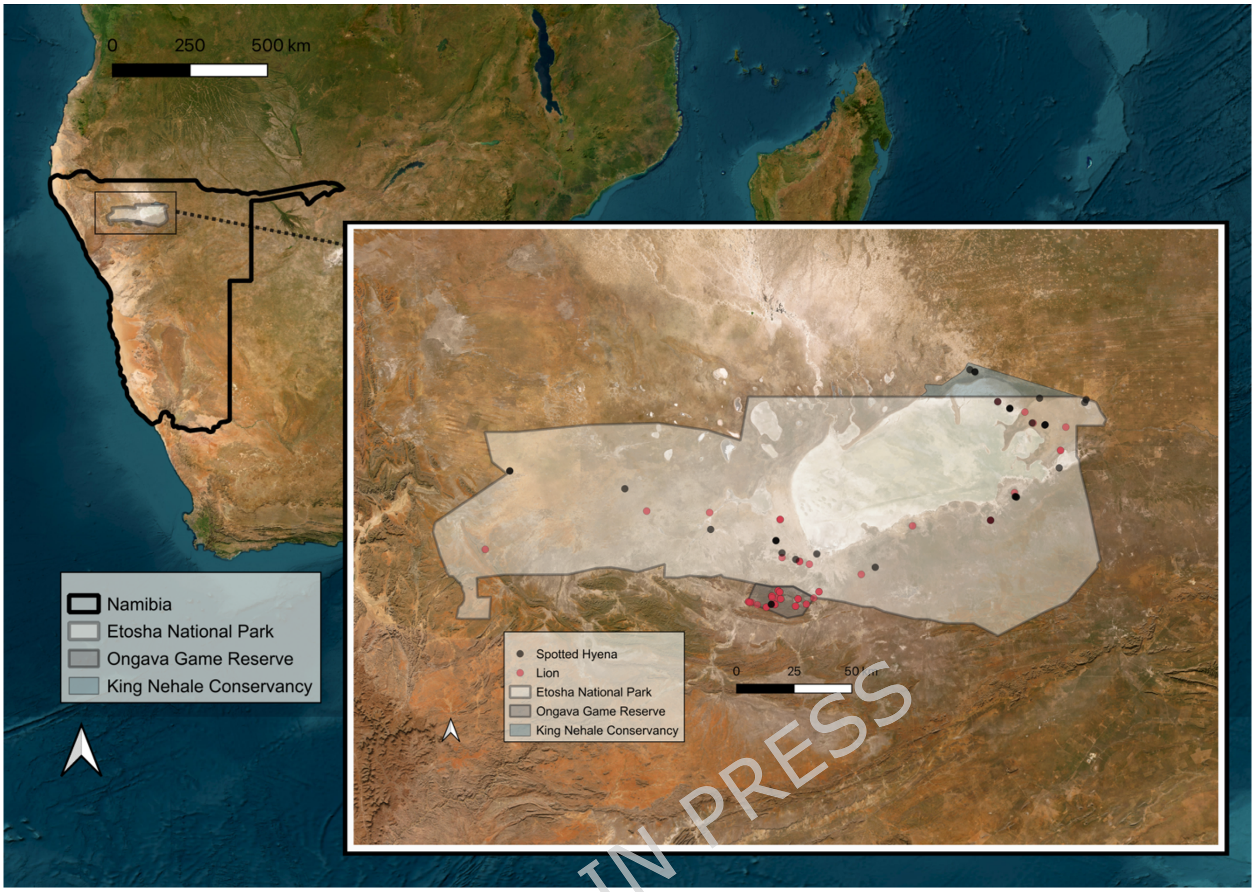

0
250
500 km
Namibia
Etosha National Park
Ongava Game Reserve
King Nehale Conservancy
Spotted Hyena
Lion
Etosha National Park
Ongava Game Reserve
King Nehale Conservancy
0
25
50 km

Fig. 1

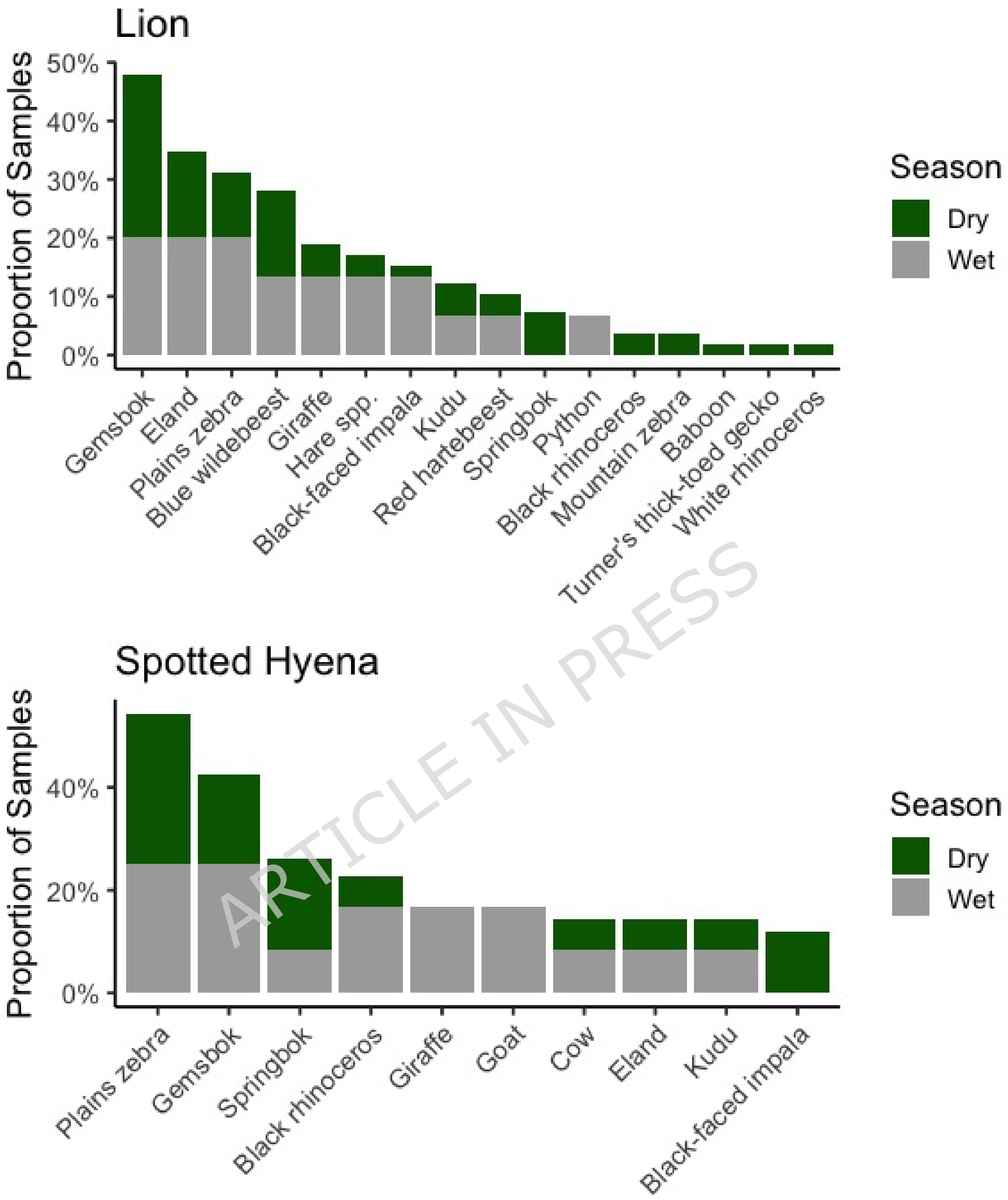

Lion
Proportion of Samples
50%
40%
30%
20%
10%
0%
Gemsbok
Eland
Plains zebra
Blue wildebeest
Giraffe
Hare spp.
Black-faced impala
Kudu
Red hartebeest
Springbok
Python
Black rhinoceros
Mountain zebra
Baboon
Turner's thick-toed gecko
White rhinoceros
Season
Dry
Wet
Spotted Hyena
Proportion of Samples
40%
20%
0%
Plains zebra
Gemsbok
Springbok
Black rhinoceros
Giraffe
Goat
Cow
Eland
Kudu
Black-faced impala
Season
Dry
Wet

Fig. 2

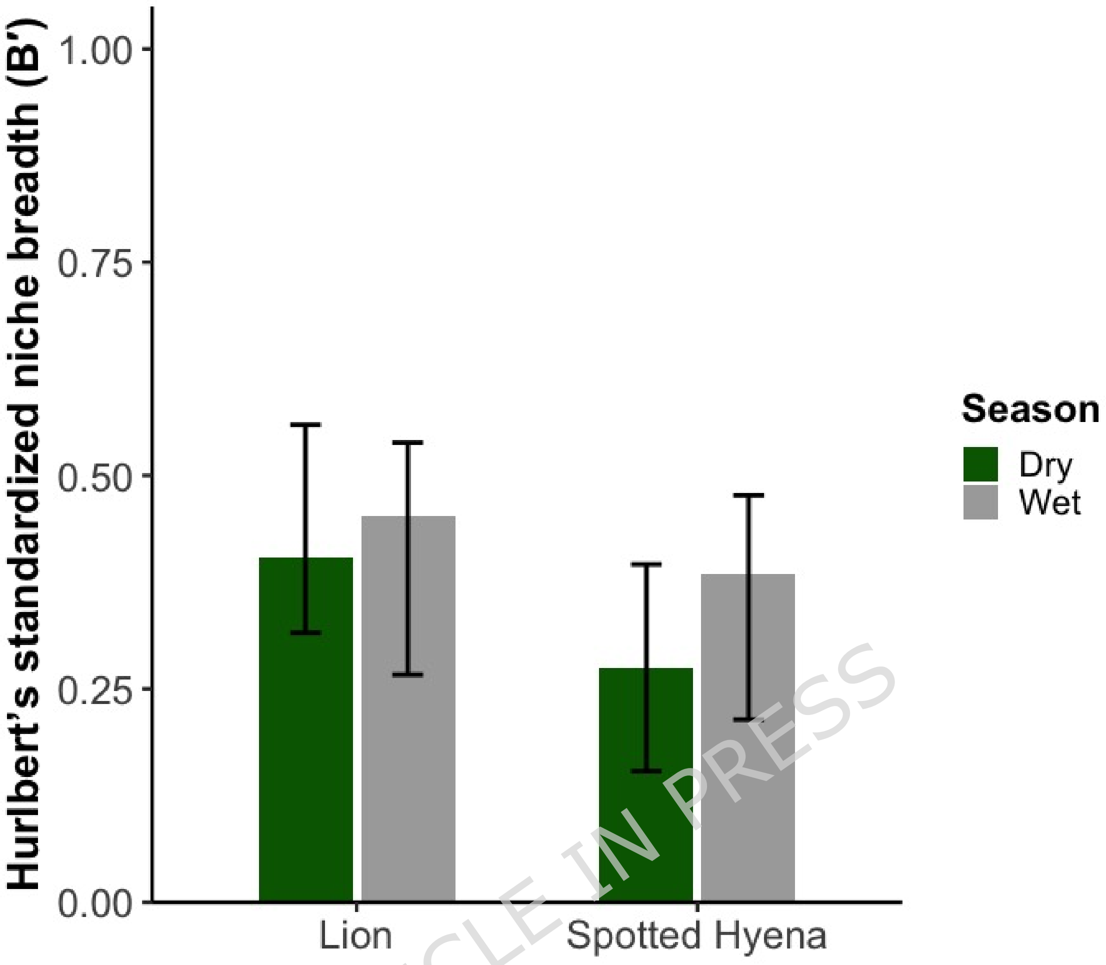

Hurlbert's standardized niche breadth (B')
1.00
0.75
0.50
0.25
0.00
Lion
Spotted Hyena
Season
Dry
Wet

Fig. 3

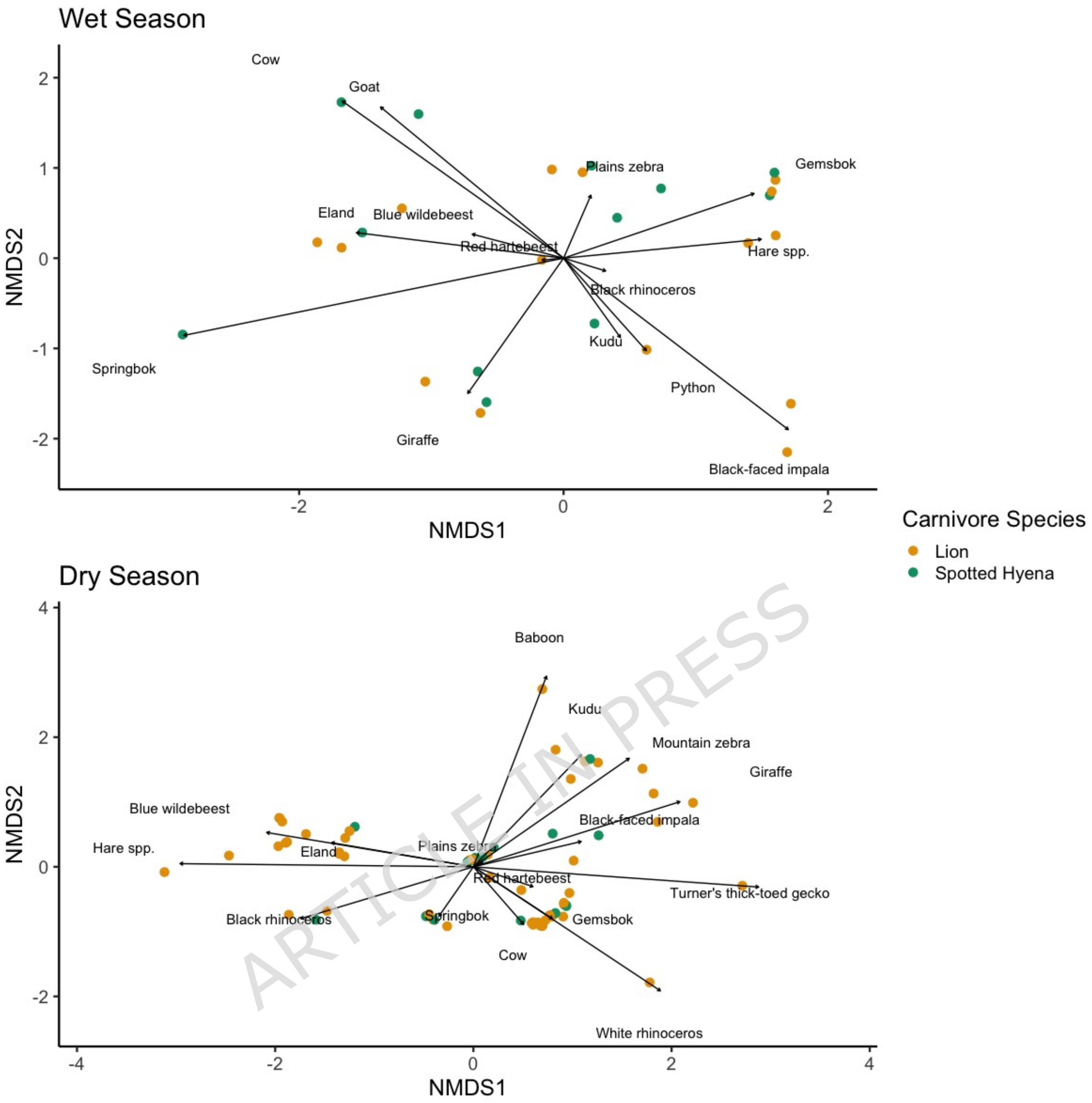

Wet Season
Cow
Goat
Plains zebra
Gemsbok
Eland
Blue wildebeest
Red hartebeest
Hare spp.
Black rhinoceros
Kudu
Springbok
Python
Giraffe
Black-faced impala
NMDS2
NMDS1
2
1
0
-1
-2
-2
0
2
Carnivore Species
● Lion
● Spotted Hyena
Dry Season
Baboon
Kudu
Mountain zebra
Giraffe
Blue wildebeest
Black-faced impala
Hare spp.
Plains zebra
Eland
Red hartebeest
Turner's thick-toed gecko
Black rhinoceros
Springbok
Gemsbok
Cow
White rhinoceros
NMDS2
NMDS1
4
2
0
-2
-4
-2
0
2
4

Fig. 4

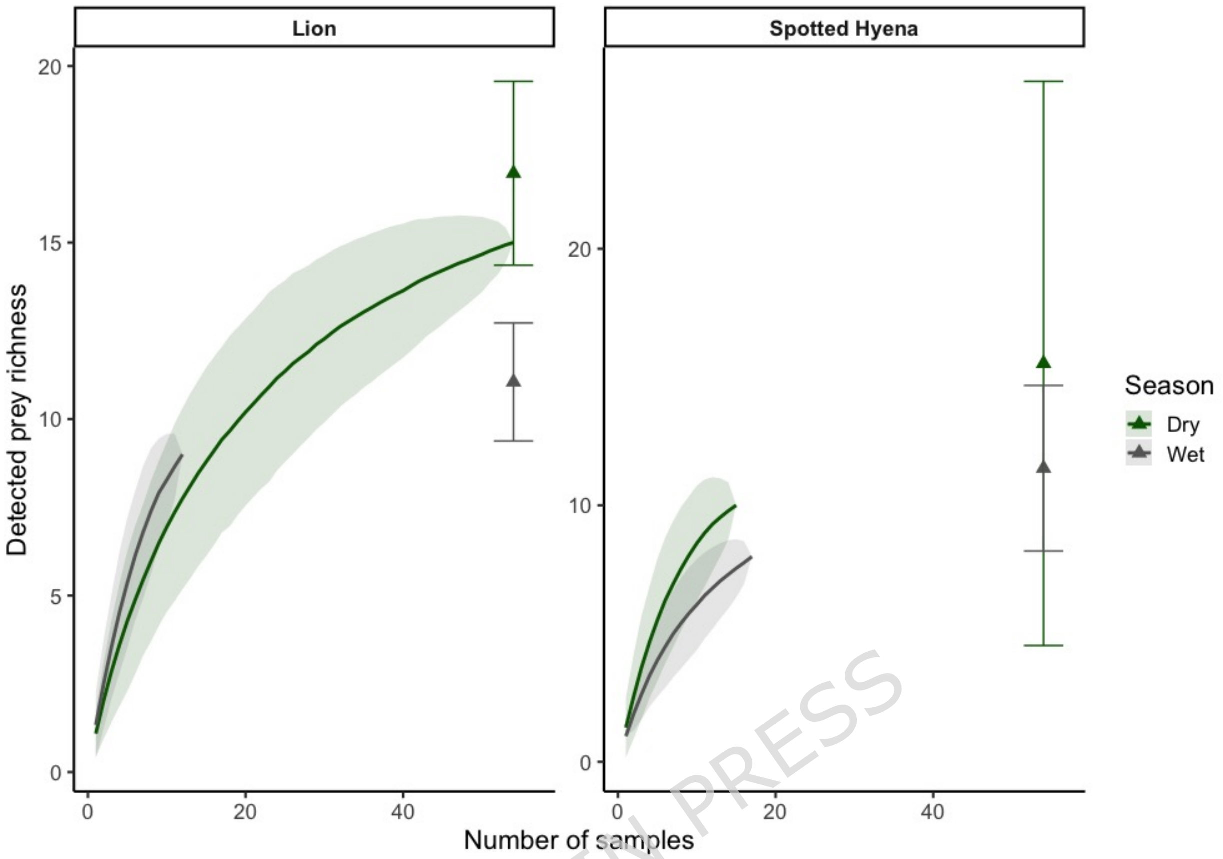


Fig. 5

Table 1 Percent frequency of occurrence (%FOO; number of fecal samples containing each prey species divided by total number of fecal samples and expressed as a percentage) and Relative Read Abundance (RRA%; total number of reads of each vertebrate prey species divided by total number of vertebrate reads and expressed as a percentage) of vertebrate species (12S rRNA) detected in the diet of African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) based on season (dry = May-October; wet =

November-April) in the Greater Etosha Landscape; “n” represents number of samples containing that prey species for that carnivore species

Carnivore - Season	Prey Species	n	Average RRA (%)	FOO (%)
Lion – Dry	Baboon	1	7.63	1.69
	Black rhinoceros	2	7.63	3.39
	Black-faced impala	1	4.99	1.69
	Blue wildebeest	8	7.32	13.56
	Common eland	8	6.86	13.56
	Gemsbok	15	7.37	25.42
	Angolan giraffe	3	7.08	5.08
	Hare spp.	2	4.29	3.39
	Greater kudu	3	7.63	5.08
	Mountain zebra	2	7.63	3.39
	Plains zebra	6	6.42	10.17
	Red hartebeest	2	3.95	3.39
	Springbok	4	5.93	6.78
	Turner's thick-toed gecko	1	7.63	1.69
	White rhinoceros	1	7.63	1.69
	Black-faced impala	2	14.46	10
	Blue wildebeest	2	3.94	10
	Common eland	3	12.01	15

Lion – Wet	Gemsbok	3	13.90	15
	Angolan giraffe	2	14.46	10
	Hare spp.	2	7.32	10
	Greater kudu	1	9.75	5
	Plains zebra	3	9.76	15
	Python spp.	1	4.71	5
	Red hartebeest	1	9.69	5
Spotted Hyena - Dry	Black rhinoceros	1	12.5	5.88
	Black-faced impala	2	12.5	11.76
	Cow	1	12.5	5.88
	Common eland	1	12.5	5.88
	Gemsbok	3	12.5	17.65
	Greater kudu	1	12.5	5.88
	Plains zebra	5	12.5	29.41
	Springbok	3	12.5	17.65
Spotted Hyena - Wet	Black rhinoceros	2	3.09	12.5
	Cow	1	0.24	6.25
	Common eland	1	15.82	6.25
	Gemsbok	3	12.1	18.75
	Angolan giraffe	2	13.07	12.5
	Goat	2	15.7	12.5
	Greater kudu	1	10.04	6.25
	Plains zebra	3	14.13	18.75

Springbok	1	15.82	6.25

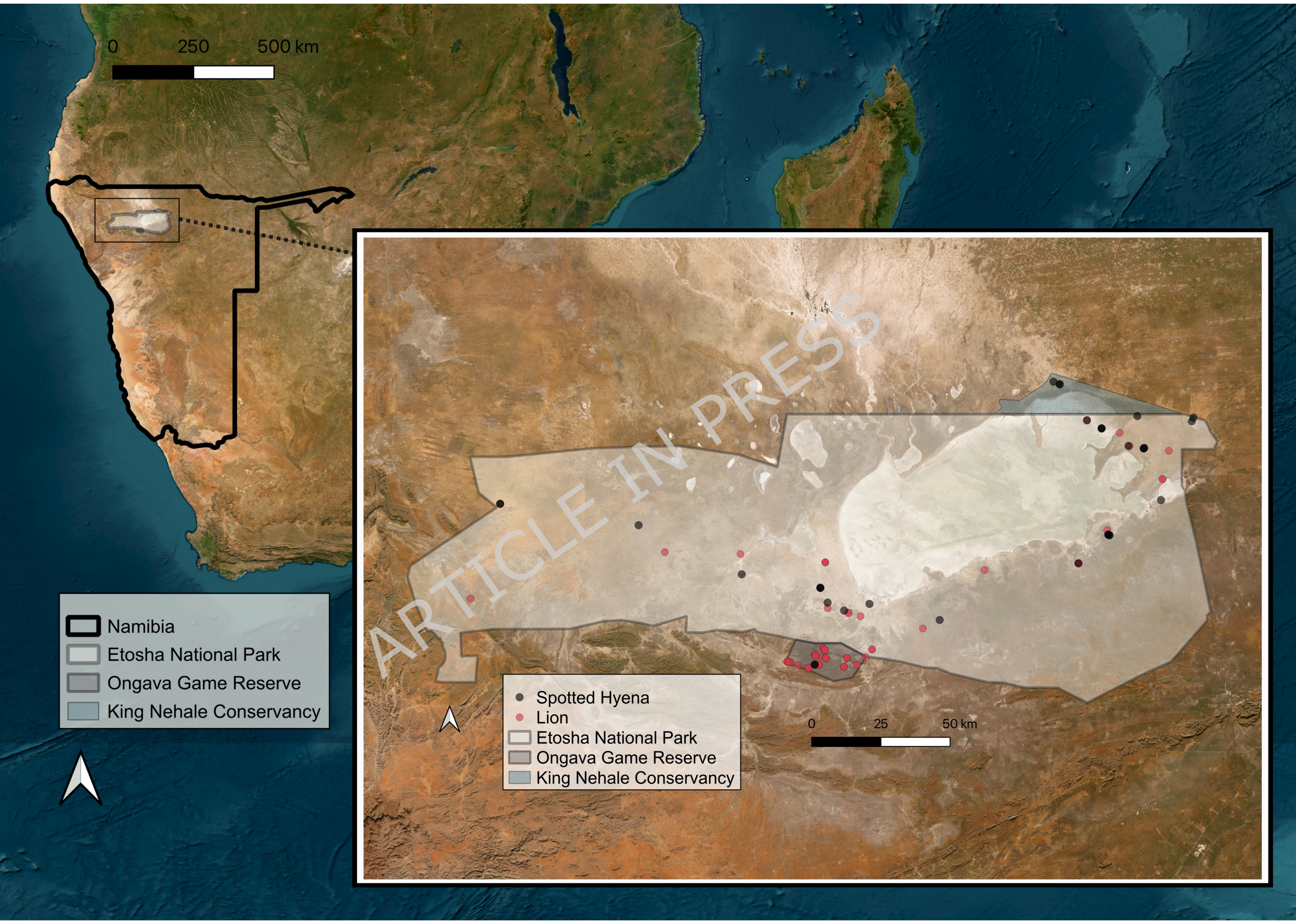
Table 2 ANOSIM results comparing prey composition between African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) in the Greater Etosha Landscape. Comparisons include between seasons (wet = November – April; dry = May – October) within each species, seasons between species, and overall between species across all samples. R-statistic and p-values are provided for each comparison

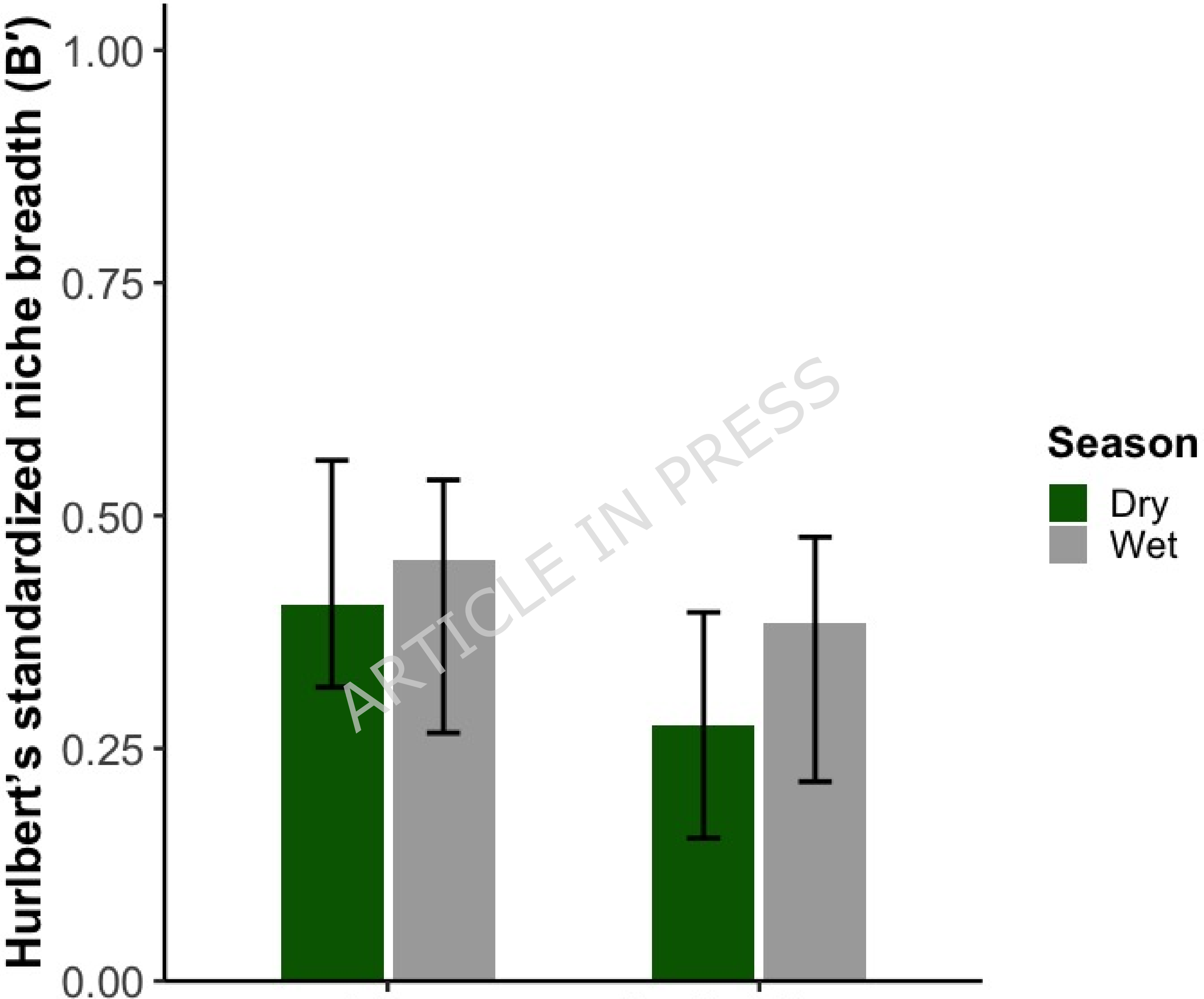
Seasonality within species (wet vs.dry)		
	R-stat	**p-value**
Spotted Hyena	-0.024	0.73
Lion	-0.008	0.58
Seasonality between species		
Dry	0.021	0.17
Wet	-0.018	0.67
Overall between species		
Lion vs. Spotted Hyena	0.014	0.17

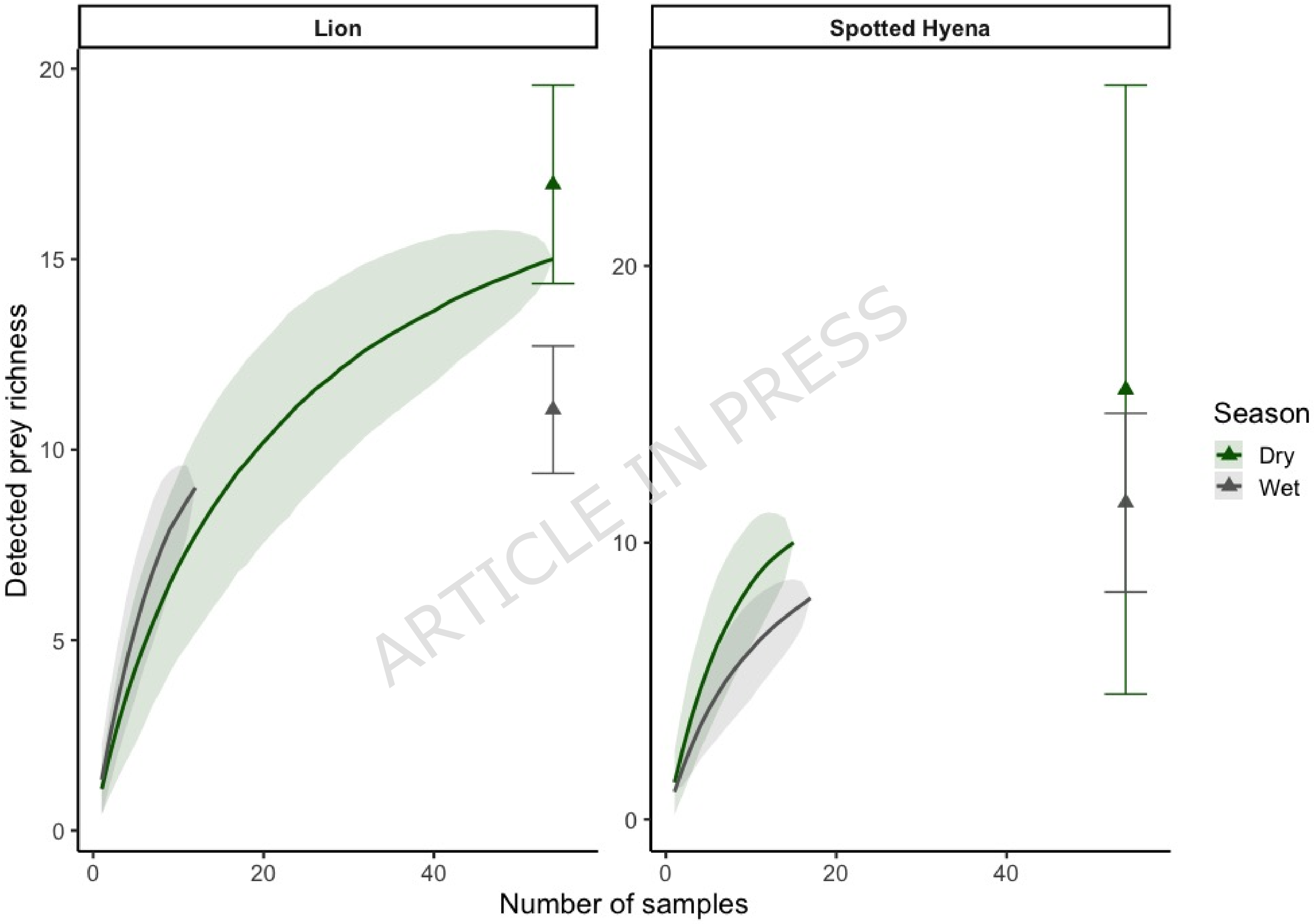
Table 3 Pianka's index of dietary overlap (O) between African lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*). Overlap values are shown with 95% confidence intervals (CIs) based on bootstrap resampling (2,000 iterations). Results are presented for seasonal comparisons within species (wet vs. dry), between species within each season, and overall between species across all samples

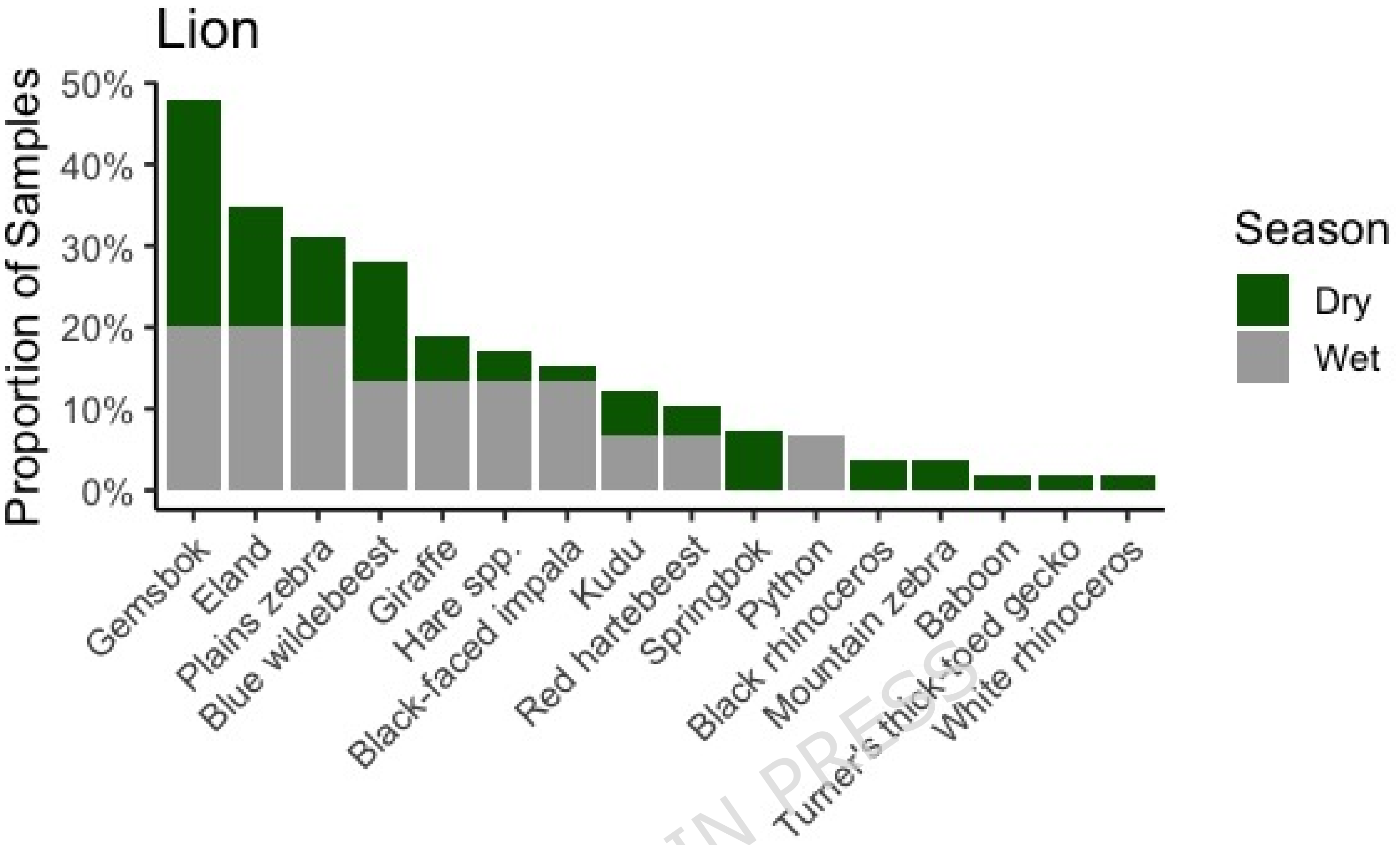
Seasonality within species (wet vs.dry)

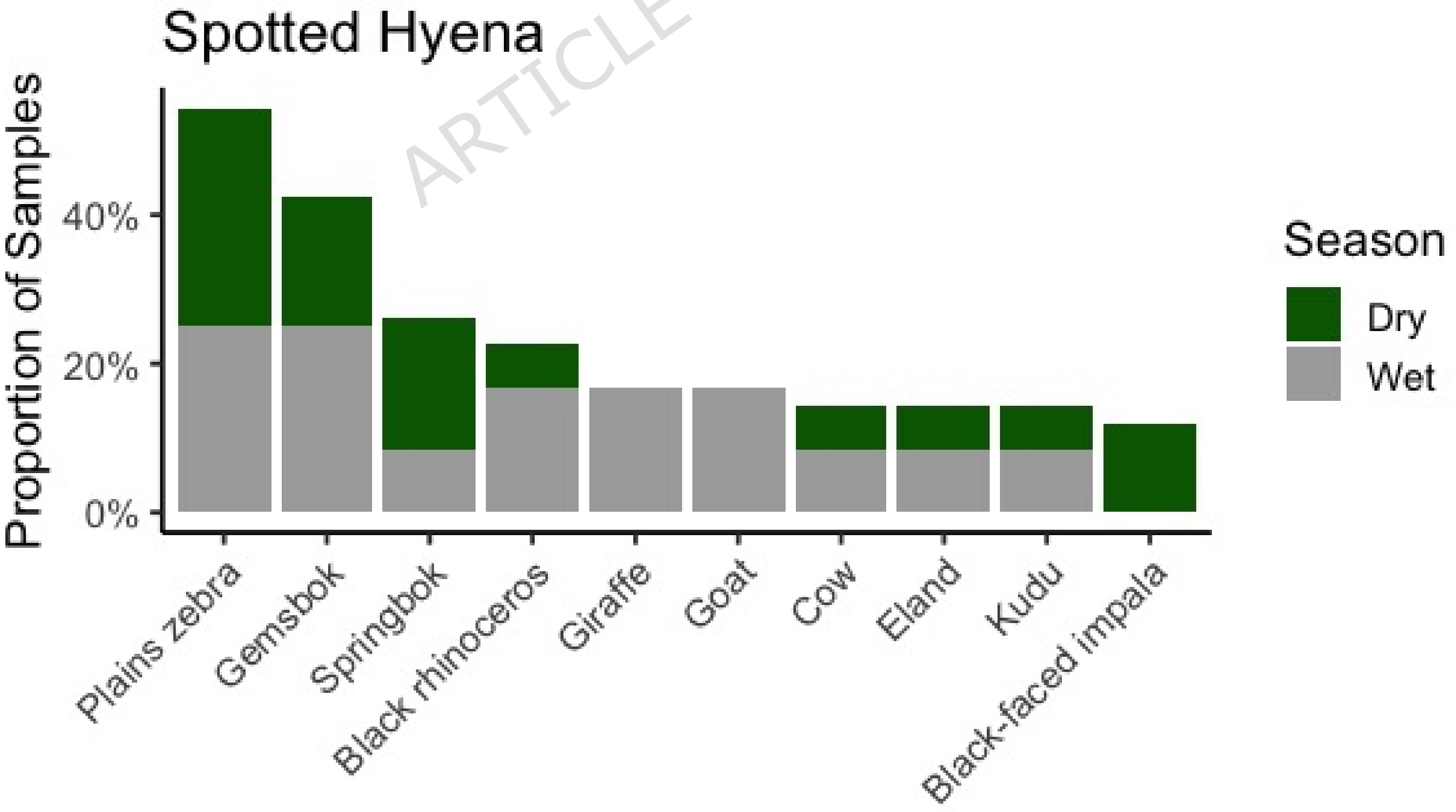
	O	**95% CI**
Spotted Hyena	0.77	0.34-0.85
Lion	0.84	0.53-0.88
Seasonality between species		
Dry	0.68	0.36-0.80
Wet	0.66	0.31-0.74
Overall between species		
Lion vs. Spotted Hyena	0.75	0.54-0.83

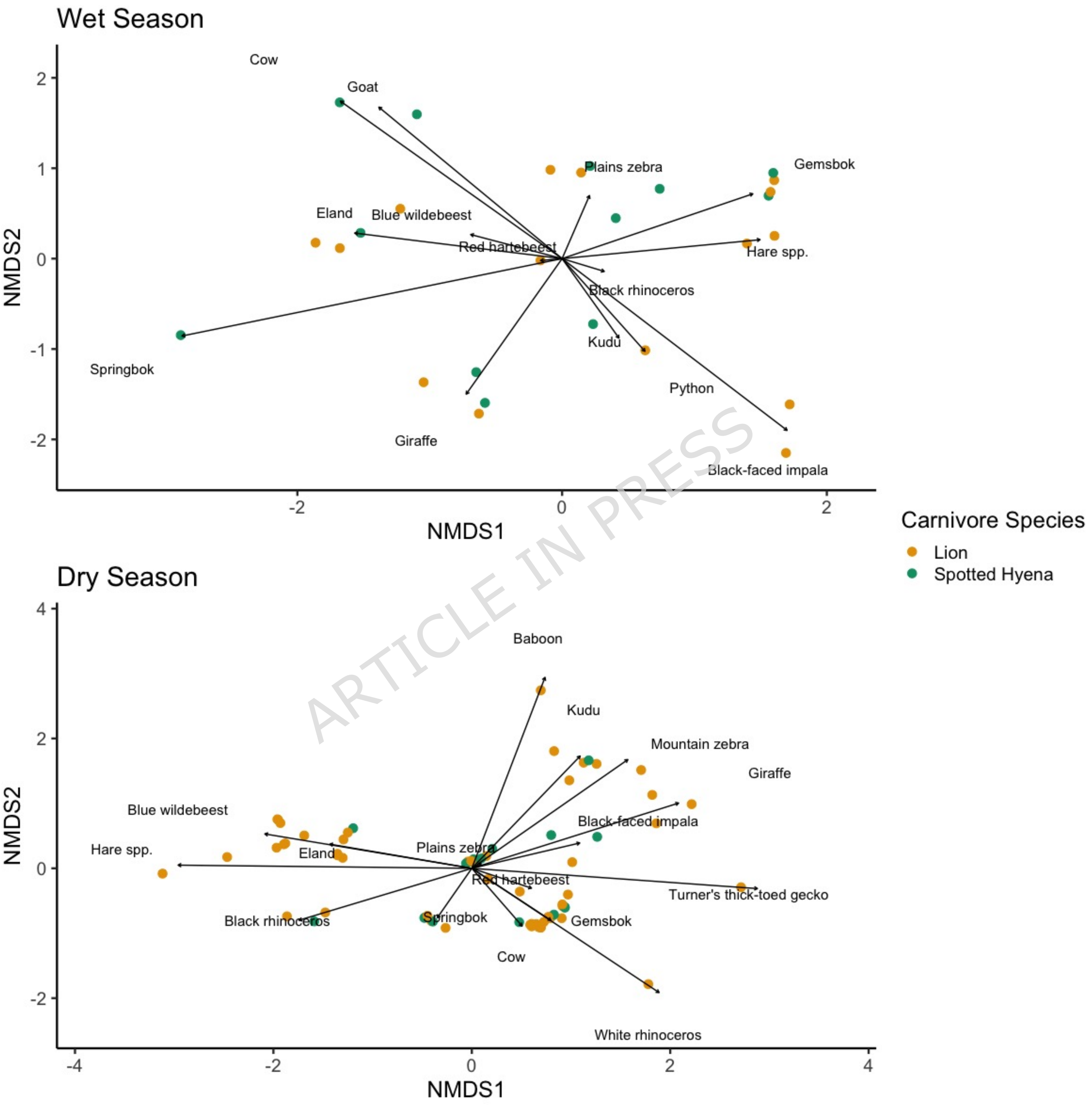

0
250
500 km
Namibia
Etosha National Park
Ongava Game Reserve
King Nehale Conservancy
Spotted Hyena
Lion
Etosha National Park
Ongava Game Reserve
King Nehale Conservancy
0
25
50 km


Hurlbert's standardized niche breadth (B')
1.00
0.75
0.50
0.25
0.00
Lion
Spotted Hyena
Season
Dry
Wet


Lion
Spotted Hyena
Detected prey richness
0
5
10
15
20
0
10
20
0
20
40
0
20
40
Number of samples
Season
Dry
Wet


Lion
Proportion of Samples
50%
40%
30%
20%
10%
0%
Gemsbok
Eland
Plains zebra
Blue wildebeest
Giraffe
Hare spp.
Black-faced impala
Kudu
Red hartebeest
Springbok
Python
Black rhinoceros
Mountain zebra
Baboon
Turner's thick-toed gecko
White rhinoceros
Season
Dry
Wet


Spotted Hyena
Proportion of Samples
40%
20%
0%
Plains zebra
Gemsbok
Springbok
Black rhinoceros
Giraffe
Goat
Cow
Eland
Kudu
Black-faced impala
Season
Dry
Wet


Wet Season
NMDS2
NMDS1
2
1
0
-1
-2
-2
0
2
Cow
Goat
Plains zebra
Gemsbok
Eland
Blue wildebeest
Red hartebeest
Hare spp.
Black rhinoceros
Kudu
Springbok
Python
Giraffe
Black-faced impala
Dry Season
NMDS2
NMDS1
4
2
0
-2
-4
-2
0
2
4
Baboon
Kudu
Mountain zebra
Giraffe
Blue wildebeest
Black-faced impala
Plains zebra
Hare spp.
Eland
Red hartebeest
Turner's thick-toed gecko
Black rhinoceros
Springbok
Gemsbok
Cow
White rhinoceros
Carnivore Species
● Lion
● Spotted Hyena

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	Gemsbok	3	13.90	15
	Angolan giraffe	2	14.46	10
	Hare spp.	2	7.32	10

Wet	Greater kudu	1	9.75	5
	Plains zebra	3	9.76	15
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